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Asymmetric and Highly Stereoselective Synthesis of the DEF-Ring Moiety of (−)-FR182877 and Its Derivative Inducing Mitotic Arrest

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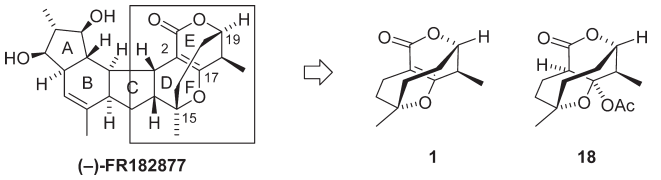
ABSTRACT



The asymmetric and highly stereoselective synthesis of compound 1, which corresponds exactly to the DEF-ring moiety of (−)-FR182877, and the biological activities of its derivatives are described. All derivatives of 1 showed no activity in the tubulin polymerization assay, but one derivative was shown to have the ability to induce mitotic arrest by interfering with microtubule dynamics, and the cellular effects are similar to those of paclitaxel.

(−)-FR182877[1a−d] and its congener, (−)-FR182876[1e] (Figure 1), were isolated from *Streptomyces* sp. No.9885 by the research group at the Fujisawa (now Astellas) Pharmaceutical Co. These compounds bind and stabilize microtubules and exhibit potent cytotoxic activity toward a number of human cancer cell lines, with a potency comparable to that of taxol. For example, the IC_{50} value of (−)-FR182877 toward P388 is 21 ng/mL.[1] (−)-FR182877 possesses a unique hexacyclic structure with 12 contiguous stereogenic centers and features a highly distorted and reactive push−pull alkene.[1c]

The notable biological activity and the extraordinary structure of (−)-FR182877 have attracted considerable attention from many synthetic chemists, and a number of synthetic studies and total syntheses,[2−5] as well as chemical biology studies, have been reported.[6]

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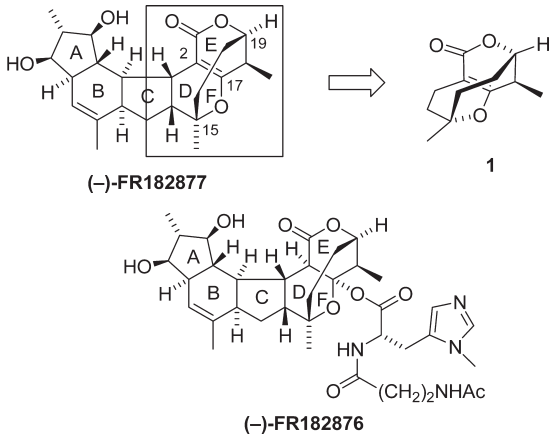


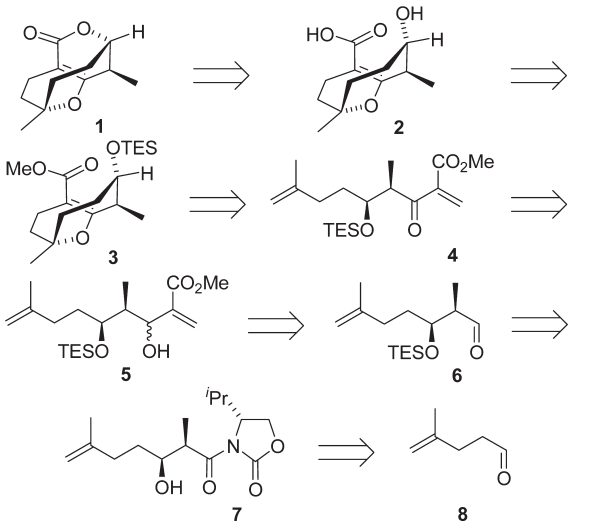
Figure 1. Structures of (−)-FR182877, (−)-FR182876, and **1**.

We recently accomplished the asymmetric total synthesis of (−)-FR182877,[4e] and during the course of that research, we became interested in the biological activity of the highly strained DEF-ring moiety of (−)-FR182877. Accordingly, we started the synthesis of compound **1** (Figure 1), which corresponds exactly to the DEF-ring moiety of (−)-FR182877, to evaluate its biological activity. We herein report the asymmetric and highly stereoselective synthesis of compound **1** via an inverse-electron-demand intramolecular hetero-Diels−Alder (IMHDA) reaction and the biological activities of its derivatives.

The DEF-ring moiety of (−)-FR182877 is highly strained as a result of the ethylene bridge between C15 and C19, and consequently, the C2−C17 alkene of (−)-FR182877 is extremely distorted and reactive. The C2−C17 alkene is easily oxidized in air to afford the corresponding epoxide,[1c] making (−)-FR182877 unstable. The E-ring of (−)-FR182877 was therefore constructed in the late stages of the total synthesis to furnish the strained polycyclic ring system in all three reported total syntheses. In view of this, it is rational to synthesize compound **1** by lactone formation of **2** (Scheme 1) in the last stage. Compound **2** was expected to be obtained from **3**, which could be prepared by the inverse-electron-demand IMHDA reaction of **4**. The inverse-electron-demand IMHDA reaction of **4** was challenging, and this type of intramolecular cycloaddition has never been previously reported.[7] We expected that the reaction would proceed because the electron-withdrawing ester group and the electron-donating methyl group that were attached at the appropriate positions of the oxa-butadiene and the terminal alkene, respectively, make the coefficients of the LUMO and HOMO suitable for accelerating the inverse-electron-demand IMHDA reaction. We thought that compound **4** could be prepared by oxidation of **5**, which could be derived from **6**. Aldehyde **6** could be prepared via **7**, which could be obtained by the Evans aldol reaction of aldehyde **8**.

(7) For the related transannular Diels−Alder reaction, see refs 2 and 3.

Scheme 1



Aldehyde **8**, which was easily prepared in three steps from a commercially available material,[8] was subjected to an Evans aldol reaction with imide **9** to afford compound **7** (Scheme 2). Compound **7** was converted to the Weireb amide **10** by a known procedure, followed by protection of the hydroxyl as a TES ether. The DIBAL-H reduction of **10** gave aldehyde **6**, and subsequent reaction with an aluminum reagent, which was generated in situ using methyl propiolate and DIBAL-H,[9] afforded **5**. Dess−Martin oxidation of **5** gave compound **4**. The inverse-electron-demand IMHDA reaction of **4** was carried out in toluene at 100 °C in the presence of BHT. The reaction proceeded slowly and required 4 days to complete but afforded **3** as a single isomer.

The TES group of **3** was easily removed by treatment with TBAF to afford **11** (Scheme 3). We then attempted to convert **11** to **2** under the conditions employed in the total synthesis of (−)-FR182877, but the reaction did not proceed because of the stable vinylogous carbonate system. DIBAL-H reduction of **11** afforded the corresponding diol, but it was sensitive to acid because the oxonium ion was easily formed and could be stabilized by the vinylogous system. Moreover, DIBAL-H reduction of **3** and subsequent Dess−Martin oxidation afforded **13**, but no suitable conditions for the oxidation of **13** to the corresponding carboxylic acid were identified, probably because the aldehyde in **13** could be stabilized by the vinylogous system.

Consequently, we decided to change the methyl ester of **3** to the PMB ester, which could be removed by treatment with DDQ or acid. Conversion of **3** to the corresponding PMB ester failed, but **5** was successfully converted to PMB ester **14** using Otera's catalyst[10] (Scheme 4). Dess-Martin

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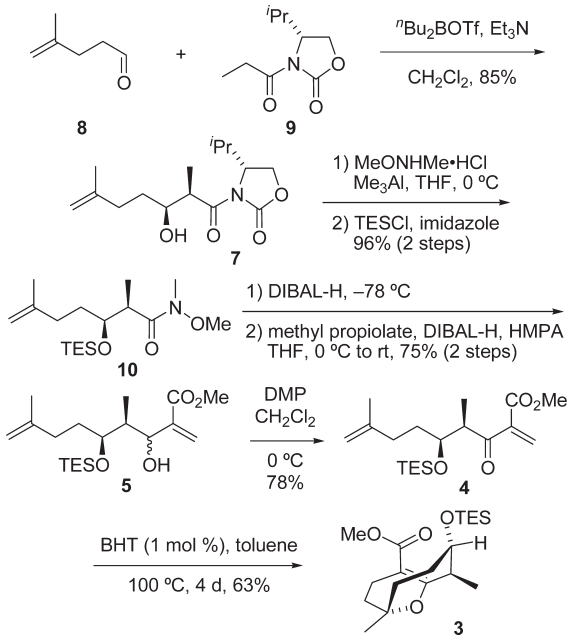
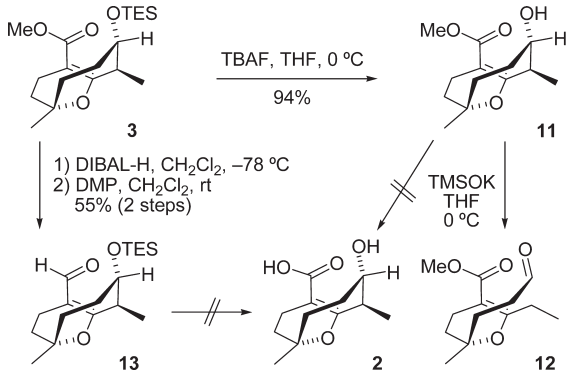
Scheme 2



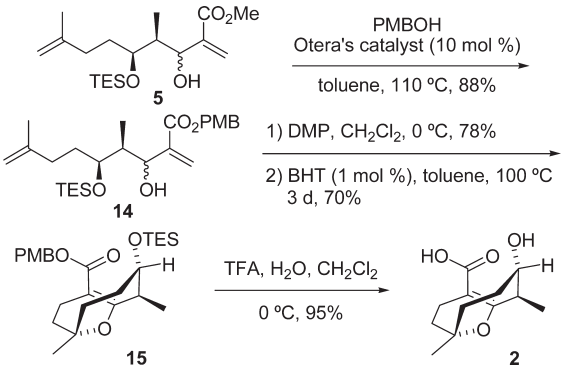
Scheme 3



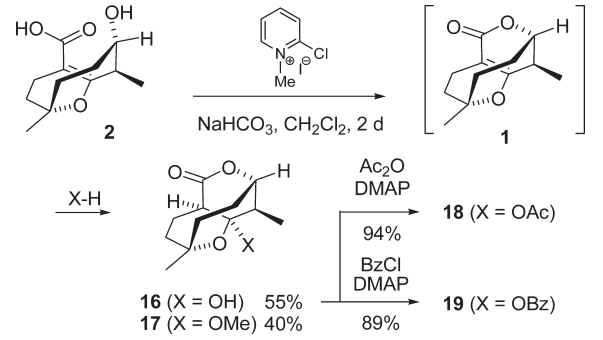
oxidation of **14** and subsequent inverse-electron-demand IMHDA reaction afforded compound **15** as a single isomer, which was successfully converted to the desired hydroxy carboxylic acid **2** by treatment with trifluoroacetic acid.

The reaction of **2** with Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide)[11] successfully afforded compound **1**[12] (Scheme 5). However, the isolated product was not **1** but a crystalline compound **16**. Recrystallization of

Scheme 4



Scheme 5



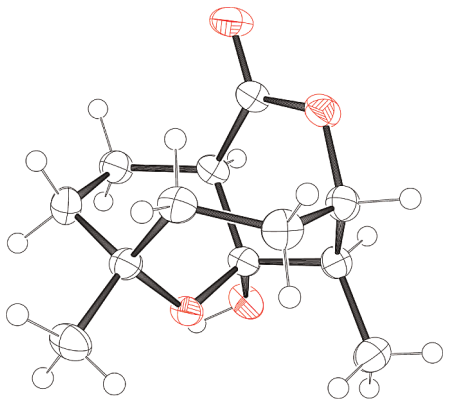


Figure 2. X-ray structure of **16**.

16 provided a single crystal suitable for the X-ray crystallographic analysis,[13] and its structure was elucidated as shown in Figure 2. Compound **16** was thought to be formed by the reaction of **1** with water during workup. Thus, compound **1** was highly reactive; the lactonization of **2** and subsequent in situ treatment with methanol easily afforded **17**. Compounds **16** and **17** were stable, and **16** was successfully converted to acetate **18** and benzoate **19**.

(11) Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49–50.

(12) Formation of **1** was confirmed by ^{1}H NMR of the crude product. See Supporting Information.

(13) Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 870027. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, U.K. [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. The atom numbers in Figure 2 do not correspond to those in Figure 1.

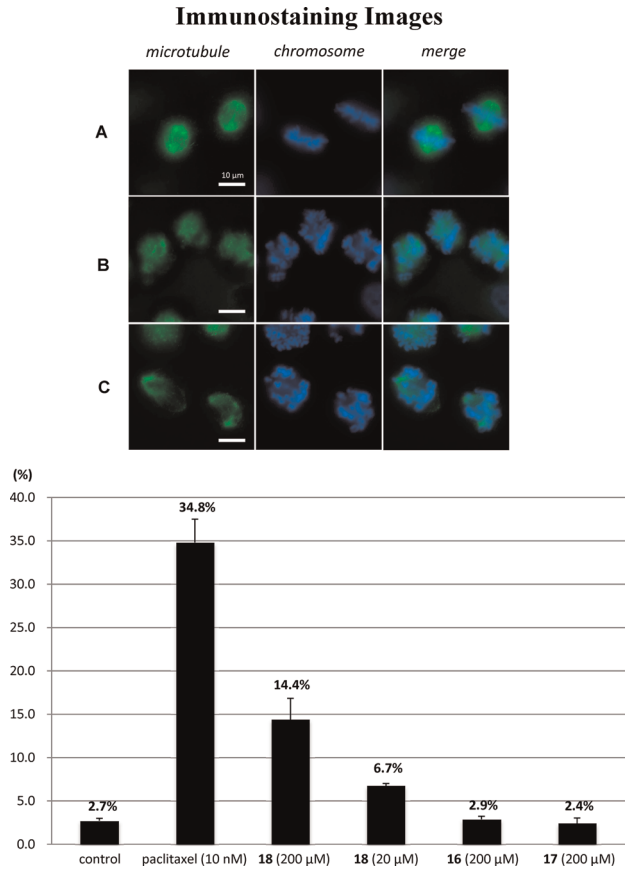


Figure 3. Immunostaining images obtained using control (A), paclitaxel (10 nM) (B), and **18** (200 μM) (C), and the mitotic indexes.

As **1** was difficult to isolate,[14] compounds **16**–**19** were subjected to the tubulin polymerization assay,[15] but interestingly, **16**–**19** showed no activity toward tubulin. We then examined cell cycle arrest with compounds **16**–**19** using HeLa cells (Figure 3).[15] Surprisingly, mitotic index analysis revealed that **18** induced cell cycle arrest in the M phase in HeLa cells within 12 h of treatment. This is consistent with the effects of paclitaxel, where disruption of microtubule dynamics prevents normal mitotic progression and leads to mitotic arrest. As shown in the immunostaining images in Figure 3, bipolar spindle formation was severely inhibited in HeLa cells after treatment with both paclitaxel (10 nM) and **18** (200 μM). These data strongly suggest that **18** has the ability to induce mitotic arrest by interfering with microtubule dynamics, and the cellular effects of **18** are similar to those of paclitaxel. Since FR182877 has been shown to covalently bind to the microtubule,[6] **18** might have a very different mode of the action. However, it cannot be ruled out that **18** is transformed to another compound in the cell, which binds to the same biding site of FR182877 or paclitaxel.

In summary, the asymmetric and highly stereoselective synthesis of compound **1**, which corresponds exactly to the DEF-ring moiety of (−)-FR182877, has been accomplished via an inverse-electron-demand IMHDA. Compound **1** was found to be highly reactive and easily afforded product **16** by an addition reaction with water at the distorted reactive alkene. Although compound **18**, the acetate of **16**, showed no activity in the tubulin polymerization assay, it induced cell cycle arrest in the M phase in HeLa cells. The activity of **18** is not as strong as that of paclitaxel, but it has been shown to have the ability to induce mitotic arrest by interfering with microtubule dynamics, and the cellular effects of **18** are similar to those of paclitaxel. Studies on the further modification of **16** and the mechanism of action of **18** are in progress.

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Supporting Information Available. Spectral data for new compounds and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

(14) Clarke et al. reported the synthesis of the compound including the DEF-ring moiety,[5c] but the ^{1}H NMR data of the product is largely different from those of compound **1** and (−)-FR182877 synthesized in our laboratory.

(15) For the experiment procedure, see Supporting Information.

The authors declare no competing financial interest.